Innovative Food Science and Emerging Technologies 37 (2016) 286–292

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Salted herring brine as a coating or additive for herring (*Clupea harengus*) products — A source of natural antioxidants?

Irene Albertos [a,*], Nina Gringer [b], Daniel Rico [a], Caroline P. Baron [b]

[a] Agrarian Technological Institute of Castilla and Leon (ITACyL), Junta de Castilla y León, Consejería de Agricultura y Ganadería, Finca Zamadueñas, Ctra. Burgos, Km 119, 47071 Valladolid, Spain
[b] Technical University of Denmark, National Food Institute, Division of Industrial Food Research, Søltoft Plads, Building 221, 2800 Kgs Lyngby, Denmark

ARTICLE INFO

Article history:
Received 31 October 2015
Received in revised form 30 August 2016
Accepted 5 September 2016
Available online 6 September 2016

Keywords:
Herring (*Clupea harengus*)
Brine
pH-shift
Glazing
Oxidation
Natural antioxidant

ABSTRACT

The objective of this study was to characterise herring brine and assess its use as natural antioxidant in herring preservation. Herring brines from different marinated products (brine from fillet-ripened spice-cured herring SC, traditional barrel-salted spice-cured herring TSp and brine from traditional barrel-salted herring TSa) were used without any pre-treatment or with a previous pH adjustment, and tested either as coating agents (glazing) for frozen herring or additives in fresh mince herring, in order to prevent oxidation.

TSa and TSp were the most effective glazing agents, retarding lipid oxidation. Brines tested as additive retarded lipid and protein oxidation in a similar trend than herring mince containing salt and/or protein. SC brine was more efficient against lipid and protein oxidation when compared to the other tested brines.

Using protein fractions isolated from herring marinating brines as glazing or additive seems feasible for preventing oxidation of both frozen and fresh herring.

1. Introduction

Barred-salted herring is an important fish product in the Nordic fishery industry whilst in the South of Europe anchovies are more common as salted product. Herring is one of the most important species in the fishing industry in Northern Europe. In 2010/2011 approximately 1.000.000 tons of herring were landed in Nordic Countries, which most ends up as salted product (Baron et al., 2015). The production of salted herring has always been of special interest in the Scandinavian countries for centuries, probably originating in the eight century (Voskresensky, 1965) as a preservation process.

During the salting process a long maturation period takes place, where degradation of proteins occurs due to both digestive and muscle proteases (Nielsen, 1995). During this long ripening period transport of biomolecules such as proteins, lipids and peptides leach out from the fish to the brine (Svensson, Nielsen, & Bro, 2004), leading to a brine rich in organic matter. After the ripening period, the maturating brine is removed and discarded and, before barred-salted herring's commercialisation, the fish is packed with fresh brine containing spices and flavourings. During the production, very large volumes of brine with high organic load are discarded. Specifically, 100 L of brines are generated per 100 kg of herring produced during the maturation step (Gringer et al., 2015). Therefore, there is a need to demonstrate if this liquid waste, which contains high-value marine biomolecules such as protein, lipids and peptides, could be re-utilised and valorised. Furthermore, marinating brine is a food grade waste and could represent a good source of natural additives with antioxidant properties. Herring brines have previously been characterised, containing proteins and peptides (Gringer, Osman, Nielsen, Undeland, & Baron, 2014) which may be able to protect lipids from oxidative damage during herring ripening (Andersen, Andersen, & Baron, 2007). Proteins might play a role in scavenging free radicals that could otherwise damage proteins. The released iron from the muscle to the brine might attack protein rather than inducing lipid oxidation in Fenton type reaction. Proteins have also been found to act as antioxidants, as they form stable and long-life protein hydroperoxides, and prevent propagation of oxidative reactions, protecting lipids from oxidative damage (Baron, Berner, Skibsted, & Refsgaard, 2005).

A recent study characterising brines from different Scandinavian products demonstrated their radical scavenging and iron chelating activities, and reducing properties using in vitro tests (Gringer et al., 2014). Similarly, a previous study showed herring press juice preventing oxidative reactions in a fish model system and a simulated gastrointestinal digestion, antioxidant capacity that was attributed to low molecular weight compounds (Sannaveerappa, Sandberg, & Undeland, 2007a; Sannaveerappa, Carlsson, Sandberg, & Undeland, 2007b). Recently, Taheri, Farvin, Jacobsen, and Baron (2014) isolated protein fractions from barrel-salted herring brines and reported that they exhibit good antioxidant properties in vitro and in simple emulsions system of 5% of fish oil in water emulsion. However, despite their potentially interesting antioxidant properties, no studies have demonstrated the valorisation of this protein-rich waste using

* Corresponding author.
E-mail address: albmunir@itacyl.es (I. Albertos).

http://dx.doi.org/10.1016/j.ifset.2016.09.008
1466-8564/

minimal and simple procedures such as one-step fractionation and centrifugation.

Glazing/Coating is a common practice in the seafood industry to preserve frozen fish from oxidation and dehydration; as alternative to this process, there is increasing research interest in edible coatings based on proteins (e.g. soy, albumin and whey), fish skin hydrolysates or chitosan (Sathivel, 2005, Sathivel, Liu, Huang, & Prinyawiwatkul, 2007, Gómez-Estaca, Montero, Gimenez, & Gómez-Guillen, 2007, Rodríguez-Turienzo et al., 2011). Kakatkar, Sherekar, and Venugopal (2004) reported that acidic fish protein dispersion applied as a glaze to frozen fish blocks or fillets had a positive impact on quality, reducing oxidation, and dehydration. Another investigation also showed that acidic dispersion of fish protein applied to seer fish (*Scomberomorus guttatus*) improved its microbiological quality compared to water glazing (Phadke, Pagarkar, Kumar Reddy, & Kumar Meena, 2012). Herring brines are dispersions of fish protein and no investigation has been performed demonstrating their ability to be used as edible coating to protect frozen fish.

The use of the pH-shift method to obtain functional proteins is one processing technology that has received a great deal of attention recently. In brief, in a first step muscle proteins are homogenised with water and solubilised at low pH (pH ≤ 3) or at high pH (pH ≥ 10.5). Centrifugation allows separation of insoluble material from the soluble myofibrillar, cytoskeletal and sarcoplasmic proteins according to Abdollahi, Marmon, Chaijan, and Undeland (2016). In a second step, the solubilised proteins are re-precipitated at their isoelectric point and a protein isolate is obtained (Undeland, Kelleher, & Hultin, 2002). This technique has been used to obtain protein isolate from several fish species including fish solid waste (Chitsomboon, Yongsawadigul, & Wiriyaphan, 2012, Park, 2012). Application of the soluble protein fraction (before precipitation) has been investigated as coating agent and several patents exist demonstrating its use as moisture retention and coating agent for preserving fish products' quality (Kelleher, 2006, 2011). However, the pH shift method has not been investigated for herring marinades effluents as a way to recover solid. Therefore, in this study brine protein was solubilised at extreme (2 or 11) pH, freeze-dried and applied to herring either as a coating agent for frozen fish or as an additive in fresh herring mince, in order to investigate possible routes for valorisation of this liquid waste rich in protein and peptides as a source of natural antioxidants.

2. Materials and methods

2.1. Brines

2.1.1. Initial brine

Brine from fillet-ripened spice-cured herring (*Clupea harengus*) (SC), traditional barrel-salted spice-cured herring (TSp) and brine from traditional barrel-salted herring (TSa) were obtained from the local fish herring processing industry (Lykkeberg A/S, Hørve, Denmark) and selected for valorisation, as shown in Table 1. TSa and TSp resulted from salting whole herring, filleting and then placing the fillets back in the brine for additional ripening, whereas SC and VC are produced as fillets or bites. Brine samples were divided into appropriate aliquots, transported on ice to the laboratory, stored at −80 °C and thawed on ice water prior to experiments.

Table 1
General composition of the three brines used.

Product	Brine	Salt	Sugar	Spice-mix	Processing time (days)
Traditional salted (Fish)	TSa	Yes	No	No	180–550
Traditional salted spice-cured (Fish)	TSp	Yes	Yes	Yes	180–550
Spice-cured (Fillets)	SC	Yes	Yes	Yes	Min 42

The pH of the raw brines was measured directly using a 780 pH meter (Metrohnm, Switzerland). All analyses on the brines were performed in triplicate and in two different sampling days.

2.1.2. Acidic and alkaline brine solutions

Fifty-millilitre samples of brines were adjusted to pH 2 or 11 (acidic or alkaline methods, respectively). All brines were centrifuged (Sorvall RC 5B Plus, Dupont, Norwalk, CT, USA) at 11.403*g* for 20 min at 10 °C. Samples were filtrated through cotton and the collected permeates were freeze-dried (Heto DryWinner 8, Thermo Fisher Scientific, Loughborough, UK) or refrigerated at 4 °C until further analysis.

2.2. Characterisation of brines

2.2.1. Mass balance

The mass balance is expressed as volume yield (final volume of permeate/sample volume after adjusting pH) in percentage (%).

2.2.2. Protein and salt content

Soluble protein content (mg/mL) of the initial brine and each acidic and basic permeate was analysed by bicinchoninic acid asay (BCA kit) (Thermo Scientific Pierce®, Rockford, USA), using bovine serum albumin (BSA) as standard. This method is based on the reduction of Cu^{+2} to Cu^{+1} by protein in alkaline medium. BCA (bicinchoninic acid) is a reagent highly sensitive and selective for colorimetric detection of Cu^{+1}. Briefly, 0.1 mL of brine were mixed with 2 mL of reagent. Samples were incubated at 37 °C for 30 min in a water bath and then cooled under cold running water. The absorbance was measured at 562 nm (Shimadzu UV 160A, Kyoto, Japan).

The salt content in percentage (w:w) of the initial brine and the acidic and basic permeates was determined using the AOAC (2000).

2.2.3. Electrophoresis

The polypeptide pattern of brines was analysed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Brine samples were diluted to 1 mg/mL in 50 mM Tris buffer (pH 7.4) containing 1 mM EDTA (Merk, Darmstadt, Germany), and further mixed 1:1 (v:v) with Laemmli buffer with 10% DDT (Sigma-Aldrich, Steinheim, Germany). Subsequently, the mixture was boiled for 3 min and centrifuged for 3 min at 13.684*g* (Heraeus™ Biofuge™ Pico, Kendro, UK). Samples and See-Blue Standard (10 μL) were loaded onto a 10% NuPAGE® Bis-Tris Gels (Novex®, 1.0 mm, 12 well, Life Technologies™, Denmark) and run with MOPS (4-morpholinepropanesulfonic acid) running buffer at 200 V for approximately 50 min). Finally, the gels were stained with Coomassie Brilliant blue G-250 overnight and washed with a destaining solution (15% ethanol and 5% acetic acid) until protein bands became clearly visible in a colourless gel matrix.

2.3. Storage experiments

2.3.1. Frozen herring coating

Ten kilograms of fresh herring (*Clupea harengus*) were obtained from local fishmongers, filleted with skin on and subsequently, vacuum packed and stored at −80 °C until further experiment. Coating was performed at 4 °C and frozen herring fillets were randomly allocated into three batches: initial brine SC, TSp and TSa adjusted to protein concentration of 1 mg/mL. Brief dipping the fish fillet in the fish protein solution for 10 s "three consecutive times" with 15 second intervals resulting in a thin coating layer onto the fillet surface. Controls consisted of non-coated fillets (No Coating), fillets vacuum packed at −80 °C upon arrival to the laboratory and fillets coated with water (Water). For each treatment, a minimum of 6 randomised fillets were used. Fish samples were placed in aluminium foil and stored for 4 and 10 weeks at −10 °C.